



Nitrate Elimination

Concern about elevated concentrations of nitrate in drinking water is growing, especially in rural areas where runoff from nitrate-rich fertilizers and animal manure often finds its way into the water supply. Nitrates are some of the highest volume chemicals made in the United States and are main components in fertilizers, pesticides, explosives, and a variety of other products. Researchers in Europe and the United States are now taking a "biocompatible" approach to the problem of nitrate by using naturally occurring enzymes to reduce the nitrate to nitrogen gas and water.

Enzymes, the catalysts of biochemical reactions, are proteins produced by cells. Specific enzymes may be derived from bacteria, plants, or animals. Corn seedling leaves and soil bacteria are common sources for enzymes that act on nitrate. Each enzyme is specific to a particular reaction or group of reactions and may require non-protein cofactors in order to function. Nitrate reductase belongs to a unique class of enzymes, called oxidoreductases, that transport electrons and thus oxidize or reduce the substrate, in this case, nitrate. In contrast to many chemical catalysts, enzymes function at normal pressures and temperatures.

Although nitrate in drinking water has not received the same attention as heavy metal or PCB contamination, it is a larger problem than often realized. In addition to agricultural sources, sewage treatment plants and airborne industrial pollution are adding to the burden. Recent analysis by the U.S. Geological Survey's Water Resources Division found that 9% of residential wells in farming areas contain nitrate levels exceeding the U.S. Public Health Service limit of 10 milligrams of nitrate per liter of water.

Surface water and deep wells have not been affected as much, but nitrate levels in

residential water are an immediate health concern for infants and pregnant women. Since the late 1940s, high levels of nitrate in drinking water have been linked to methemoglobinemia or "blue baby" syndrome, in which the oxygen-carrying capacity of an infant's blood is greatly reduced, sometimes leading to death. A link to some types of cancer is also suspected, although unproven.

Contamination of drinking water with nitrate is a long-term problem because once nitrate reaches groundwater, natural processes are very slow in removing it. Most commonly used nitrate removal systems have been based on ion exchange, reverse osmosis, or electrodialysis—processes that do not degrade the nitrate and thus do not entirely solve the problem. Other systems under development rely on microorganisms to degrade the nitrate, but the process is slow, unstable, and may contaminate the water with other substances. Researchers are hoping that an enzyme-based system will be more effective and less expensive than existing systems.

Made in Germany

MoBiTec GmbH, a small company in Göttingen, Germany, has developed a process that quickly reduces nitrate first to nitrite, then to nitrous oxide, and finally to nitrogen gas and water by using a series of immobilized enzymes that act as cofactors. Nothing else is added to the water, and no residues are produced.

Within a reaction chamber, the enzymes and dyes that act as cofactors are immobilized in thin layers of a polymer matrix. These are attached to the surface of a low-voltage cathode. Nitrate-laden water (the substrate) is then passed through the matrix and nitrate reduction begins. In effect, this method resembles a microorganism-based reduction system, but enzymes and cofactors are used rather than

bacteria. Electricity is the source of energy used to complete the chemical reaction, which is much faster than relying on cellular processes.

According to Stephan Diekmann, owner of MoBiTec, the idea for this system came from his colleague Robert Mellor, who worked with the company from 1988 to 1991 with the support of the Germany Ministry of Research and Technology. Diekmann is also head of the molecular biology department at the Institute of Molecular Biotechnology in Jena, a small industrial city in former East Germany that is rapidly establishing itself as a high technology center, where much of the developmental work on the enzymes is being conducted.

The Institute for Molecular Biotechnology was once part of the scientific conglomerate ZIMET (Central Institute for Microbiology and Experimental Therapy). Now the institute uses an interdisciplinary approach to change chemical processes to biocompatible processes. Its work is grouped around biopolymer structures and engineering, evolutionary biotechnology, and molecular genome analysis. Diekmann's molecular biology department is especially interested in producing enzymes in large and highly reproducible quantities for use as tools.

"We want to understand how enzymes work," says Diekmann. "And then design our own as small and effective as they can be. If you want to use such enzymes as active catalysts, you want to keep them immobilized."

"We have demonstrated the proof of principle for our bioreactor," Diekmann continues. "Now we must really understand and measure the exact molecular arrangements of the enzymes." For this, the researchers use spectroscopy, plasma resonance imaging, and atomic force microscopy.

Joseph Tart

Diekmann's group is currently experimenting with several different bacteria, including *Pseudomonas stutzeri* and *Alcaligenes eutrophus*, to express the enzymes and then investigate their potential for large-scale and inexpensive production. He plans to have the nitrate and nitrite reductases available by the end of 1996. A working prototype of the bioreactor should be ready the following year. Diekmann calculates that the process will be half as expensive as reverse osmosis for nitrate removal from drinking water.

"We still have many questions, such as how pure must the enzymes be and what is the optimal design [of the bioreactor] for high flow rates," says Diekmann. MoBiTec is now working on several variations of the reactor design. Diekmann believes that to meet drinking water standards in the future, water will have to be denitrified on an industrial scale. A city of 100,000, like Jena, would need a reactor of one cubic meter.

Diekmann concludes, "In principle, this technique can be used for water purification with any kind of substrate—for example, pesticides—when the degradation process and correct degrading enzymes are known."

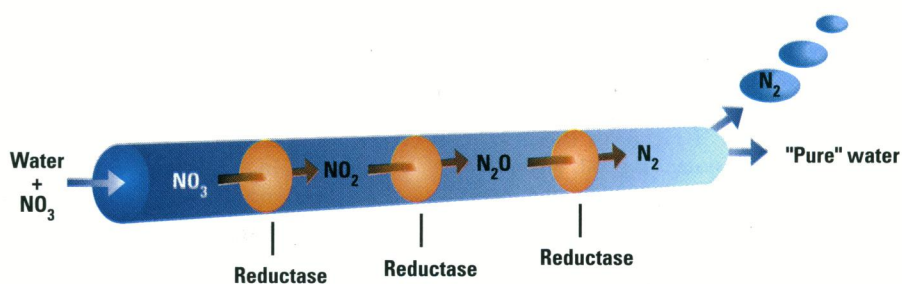
Northwest Passage

In the United States, a small company in Michigan is working on an application of this enzyme technique to remove nitrate from drinking water at or near the faucet. The Nitrate Elimination Company, Inc. (NECi) is beginning phase II of an EPA Small Business Innovation Research (SBIR) grant to complete development of its Enzymatic Nitrate Elimination Technology (EzNET) system for small water systems. The company's president, Wilbur Campbell, is also director of the Phyto-technology Research Center at Michigan Technology University in Houghton.

"We are concentrating on the practical side of the technology to make a system that is safe, inexpensive, and very easy to install and use," Campbell says. Campbell is a specialist on nitrate reductase in plants, in which it affects the growth cycle.

NECi currently uses a nitrite reductase and a nitrous oxide reductase derived from the common soil bacterium *Rhodobacter spheroides forma denitrificans*. All the enzymes and cofactors are immobilized on an inorganic material used in the food processing industry. The system can be visualized as a column, with the three enzymes in a series energized by a direct low-voltage current.

In phase I of the SBIR grant, NECi demonstrated that, on a single pass, the



Chain of tools. As contaminated water moves through the bioreactor, reductases convert nitrate to nitrite, then nitrite to nitrous oxide, and finally nitrous oxide to nitrogen gas and water. (Source: EzNET)

EzNET system works for at least 4 days at room temperature on local tap water spiked with 10 parts per million nitrate. In that time, the system removed 85% of the nitrate. The primary technical barriers to commercialization are the stability of the enzymes in actual situations and a cost-effective reactor design.

Campbell expects that the two-year time frame of phase II will result in a viable commercial design. This design would permit EzNET to be a stand-alone system if a high nitrate level is the only problem in a home, or to work as an add-on component to water softening or filtration systems. If a module of EzNET enzymes can be made to last 3 months, then the annual maintenance cost would be about \$80, in addition to an installation cost of perhaps \$200. Existing home water systems that use reverse osmosis or distillation can remove nitrate along with other contaminants but are less efficient and more expensive to install and operate.

Cautious Optimism

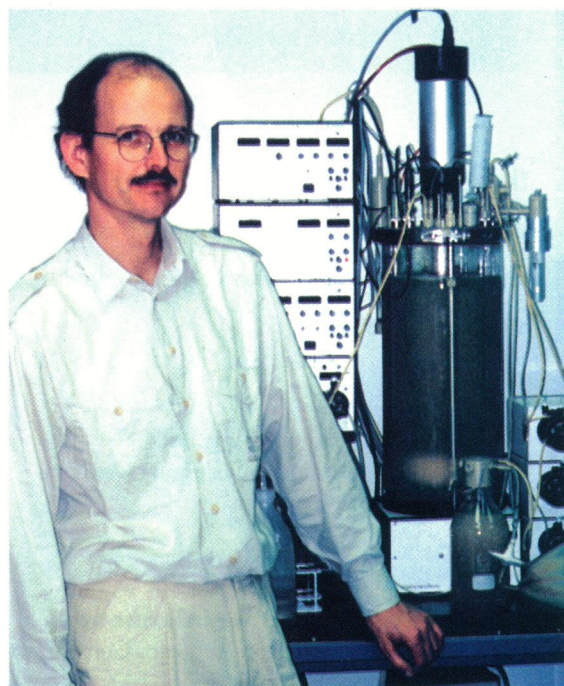
The concept of enzymatic removal of nitrate from drinking water seems like a good idea to others in the field of combating contamination, but there are outstanding questions to be resolved. William Suk, chief of the Chemical Exposures and Molecular Biology Branch of the NIEHS's Division of Extramural Research, wonders how the concept will function in the real world. "What will prevent other chemicals or microbes in the water from degrading the enzymes, and, if it's only enzymatically driven, can it process water fast enough to be practical?" asks Suk.

These are legitimate questions that the developers are try-

ing to answer by using enzymes that are very stable when immobilized and that can denitrify water essentially instantaneously. A system may also need to include some sort of pre-filter, although water from a public water system should not be a problem for the enzymes, and deep well water is relatively free of microbes.

Simeon Komisar, an assistant professor of environmental engineering at Rensselaer Polytechnic Institute, sees this kind of technology as part of a biotechnological approach to the environment that will find increasing use in the coming decades. "The genius of this concept is the way in which they've immobilized the enzymes in a semipermeable matrix and can apply a low-voltage current. They've eliminated the risk associated with microorganisms and increased the reaction rate," says Komisar.

Komisar has been awaiting the com-



Enzyme entrepreneur. Stephan Diekmann at the enzyme fermentation station, which produces the enzymes for the bioreactor.

mercialization of this technology with great interest but, like Suk, has questions. "Most real situations are not like the lab. Other substances may gum up or interfere with the reaction. If nitrate is the only problem, then this system is good, but you may need to filter out other things and the question becomes: at what expense."

Developing the practical side of this technology is the goal. The science works and has led to the development of two viable concepts for treating drinking water. As a result, other parties are beginning to take notice. One party has even proposed using the enzyme technology as part of a vacuum vaporization well for on-site remediation of nitrate contamination.

Many more developments should follow because biotechnology now offers the prospect of converting nitrate, a potentially harmful substance, into one of the most common substances on earth, nitrogen gas, using a biocompatible process.

Conard Holton

SUGGESTED READING

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